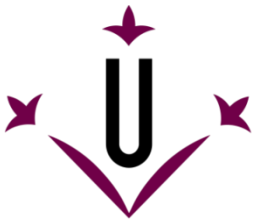

Germination behaviour of *Conyza bonariensis* to constant and alternate temperatures across different populations

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Abstract

Conyza bonariensis is one of the most problematic weed species throughout the world. It is considered highly noxious due to its interference with human activities, and especially the competition it poses with economically important crops. This research investigated the temperature requirements for seed germination of four populations of *C. bonariensis* with distinct origin and the influence of daily alternate temperatures. For this, a set of germination tests were performed in growth chambers to explore the effect of constant and alternate temperatures. Seeds of the four populations (from Lleida, Badajoz and Seville, Spain and Bahía Blanca, Argentina) were maintained at constant temperatures ranging from 5–35ºC. The final germination and cardinal temperatures (base, optimum and maximum) of each population were obtained. We also tested the influence of daily alternate temperatures on final germination. To do so, seeds were exposed to two temperature regimes: 5/15, 10/20, 15/25, 20/30 and 25/35ºC night/day temperature (intervals increasing 5ºC, with constant oscillation of 10ºC) and to 18/22, 26/24, 14/26, 12/28 and 10/30ºC night/day temperature (intervals with average of 20ºC, but increasing the oscillation in 4ºC between intervals). In general, all populations behaved similarly, with the highest germination percentages occurring in the optimum temperature range (between 21.7ºC and 22.3ºC) for both constant and alternate temperatures. In general, climatic origin affected germination response, where seeds obtained from the coldest origin exhibited the highest germination percentage at the lowest temperature assayed. In addition, we observed that the alternate temperatures can positively affect total germination, especially in oscillations that were further from the average optimum temperature (20ºC), with high germination percentage for the oscillations of 15/25, 20/30, 18/22, 16/24, 14/26, 12/28 and 10/30ºC in all populations.

The cardinal temperatures obtained were significantly different across the populations. These results provide information that will facilitate a better understanding of the behaviour of *Conyza* and improve current field emergence models.

1 | Introduction

Weed management is one of the most important issues in crop production, especially in conservation cropping systems. Some weeds are difficult to control due to their specific biological characteristics and the lack of information about them.

Conyza bonariensis (L.) Cronquist, (hairy fleabane, flaxleaf fleabane) is one of the most problematic weed species throughout the world (Bajwa et al., 2016), appearing in more than 40 crops in 70 countries (Holm et al., 1997). In Spain, it is one of the most competitive introduced noxious weeds (Zambrano-Navea et al., 2013) that harms crops and leads to yield loss (Davis & Johnson, 2008; Trezzi et al., 2013, 2015; Urbano et al., 2007). *Conyza bonariensis* is an annual or short-lived perennial weed native to South America (Thebaud & Abbott, 1995; Wu et al., 2007). Its invasive behaviour is due to high plant fecundity that varies from 85,000 (Dauer et al., 2007) to 375,500 seeds per plant (Kempen & Graf, 1981). Moreover, the anemochorous dispersion of the seeds permits their long-distance spread and establishment in new fields (Savage et al., 2014). *Conyza bonariensis* is difficult to control in minimum tillage and conservation cropping systems (Somervaille & McLennan, 2003; Wicks et al., 2000), but is easily controlled with tillage (Brown & Whitwell, 1988). Herbicide control depends on the growth stage of the plants (Shrestha et al., 2008) and if the population presents herbicide resistance, given that *C. bonariensis* has been cited as evolving resistance to herbicides with different action sites (EPSP synthase inhibitors, PSI Electron Diverter, ALS inhibitor,

etc.) in several countries (Argentina, Australia, United States or Spain), in addition to evolving multiple resistance (PSI Electron Diverter and EPSP synthase inhibitors) (Heap, 2019).

Conyza is photoblastic, emerging from the upper layers of the soil surface (0–2 cm) with limited persistence, as it has very low dormancy levels and the viability of the ungerminated seeds is severely lost in the first year (Wu et al., 2007). Annual weed species survival is highly dependent on seedling emergence and recruitment (Forcella et al., 2000). Thus, it is important to know both timing and magnitude of seedling emergence in the field in order to implement successful control measures for weeds (García et al., 2013; Royo-Esnal et al., 2015). In this respect, Zambrano-Navea et al. (2013) modelled the emergence of *C. bonariensis* and developed a cohort-based stochastic model of the population dynamics (Zambrano-Navea et al., 2016). However, studying the germination response of more populations and at more temperatures and intervals would expand upon these existing models. *Conyza bonariensis* biology is well understood, but additional information regarding germination temperature thresholds is required to implement integrated management control measures. An added complexity is that the variation of threshold parameters between populations can be significant due to local adaptations (Tozzi et al., 2014; Bajwa et al., 2016). For example, in *C. bonariensis*, Karlsson & Milberg (2007) established cardinal temperatures of 4.2ºC (base temperature, T_b), 20ºC (optimum temperature, T_o) and 35ºC (ceiling temperature, T_c), while Zambrano-Navea et al. (2013) cited a T_b of 10.6ºC.

This research investigated thermal requirements for seed germination of four different *C. bonariensis* populations collected from contrasting environments. The final aim was to compare the total germination percentage of each population at constant

temperatures and at different alternate temperatures. An additional objective was to compare the cardinal temperatures (T_b, T_o, T_c) of each population.

2 | Materials and methods

2.1 | Plant material collection

Conyza bonariensis seeds were harvested at maturity in September 2016 in Spain and in November 2017 in Argentina. Seeds from Spain were collected from three different habitats: a vineyard in Lleida (41.658010, 0.523766), a garden in Seville (37.352824, -5.933194) and an olive orchard in Badajoz (38.702537, -5.573246). The population from Argentina belonged to a garden in Bahía Blanca (-38.695394, -62.253302). The four locations have specific climatic conditions (Table 1). According with Torra et al. (2016), seeds were collected from different plants throughout the field, were air-dried under laboratory conditions for one week and dry stored in the dark in paper bags at 4ºC until the beginning of the experiment.

2.2 | Experimental design

Three germination tests were established at different temperature conditions and repeated twice: the first one at constant temperatures, the second at constant day/night temperature oscillations (T_{osc}) and different mean temperatures (T_m), and the third one at different day/night T_{osc} but with the same T_m. All the experiments were performed at the Departamento de Agronomía, Universidad Nacional del Sur and CONICET (Bahía Blanca, Argentina). In all three tests, batches of 30 seeds were sown on 9-cm Petri dishes lined with a Nº1 filter paper layer wetted with distilled water. Four replicates per population and temperature were used following a completely randomized design.

Germinated seeds were counted on a daily basis until 21 days or until no further germination occurred during five consecutive days.

2.2.1 | *Germination test at constant temperatures (Test 1):*

Seeds from each population were incubated, in November 2017, at constant temperatures of 5, 10, 15, 20, 25, 30 and 35ºC with a 12/12 day/night photoperiod. Temperatures were maintained at ±0.2ºC and monitored with digital temperature data loggers (Thermochron Ibuttons, Model DS1921G-F50; Maxim Integrated Products, Inc., San Jose, California, USA).

2.2.2 | *Germination test at alternate temperatures (variable T_m/constant T_{osc}) (Test 2):*

In this assessment, seeds from each population were incubated at different alternate temperatures with 10ºC of T_{osc} 5/15, 10/20, 15/25, 20/30 and 25/35ºC night/day temperatures using an Electronic Gradient Plate Germinator (SECELEC, CCT-Bahía Blanca, CONICET). Seeds were placed inside independent germination chambers with automatic temperature control (±0.1ºC).

2.2.3 | *Germination test at alternate temperatures (constant T_m/variable T_{osc}) (Test 3):*

In this test, T_m was maintained constant (20ºC) while T_{osc} was varied. The T_m considered was the optimum temperature (T_o) obtained by Wu et al. (2007). Seeds were then placed at constant 20ºC (in a growth chamber) and at 18/22, 16/24, 14/26, 12/28 and 10/30ºC night/day alternate temperatures. Thus, incubation temperature amplitudes (T_{osc}) were 0, 4, 8, 14 and 20°C.

For the different tests, a seed was considered germinated when the radicle had extended more than 1mm beyond the seed coat (Steinmaus et al., 2000; Wu et al., 2007).

Germinated seeds were removed from the dish once counted. Seed viability at the end of the germination tests was assessed by counting the number of germinated seeds after incubation at 20ºC (Wu et al., 2007) under a 12-h photoperiod for five days.

2.3 | Statistical analysis

Total germination percentages between populations and incubation temperatures as well as cardinal temperatures were subjected to analysis of variance (ANOVA). The SED and LSD are provided.

Estimation of cardinal temperatures (base temperature, T_b, optimum temperature, T_o and ceiling temperature, T_c)

In order to estimate the cardinal temperatures for each population, a three parameter logistic function was first fitted to the cumulative emergence of each replication of each population at each constant temperature (Eq. [1]).

$$Y = \frac{a}{1+e^{\left(\frac{-(x-d50)}{b}\right)}} \qquad \text{Eq. [1]}$$

Where Y is the germination percentage, a is the maximum germination percentage, d_{50} is the time in days to achieve 50% of germination and b is the germination rate at d_{50}.

Estimation of the optimum temperature (T_o):

Once d_{50} was defined, its inverse value ($1/d_{50}$) where represented in a figure and a three-parameter Lorentzian function was fitted, equally, to each replicate of each population (Eq. [2]).

$$Y = \frac{a}{1+\left(\frac{x-d50}{b}\right)^2} \qquad \text{Eq. [2]}$$

Where Y is $1/d_{50}$ value at each temperature x, a is the maximum$1/d_{50}$ value, x_0 is the temperature at which the highest value of $1/d_{50}$ is obtained, and coincides with the

centre of the peak and the optimum temperature at the same time; and parameter b is the mean width of the peak.

T_b and T_c estimation:

Once T_o was defined, sub-optimal temperatures were used to obtain T_b and supra-optimal temperatures were used to obtain T_c, and regression lines were fitted, respectively, to each (Eq. [2]) (Guillemin et al., 2013; Torra et al., 2016).

$$Y = ax + b \qquad \text{Eq. [3]}$$

Where Y is the $1/d_{50}$ value at each temperature x, a is the slope and b is a constant value. The point where the regression lines intercept the X axis, estimated with the mathematical approach of the regression line, was considered as the T_b and T_c respectively for each population (Holt & Orcutt, 1996; Steinmaus et al., 2000; Wu et al., 2007), assuming there are no intra-population variations.

All statistical analyses were performed with JMP Pro 14 software (SAS Institute 2010. SAS Campus Drive, Cary, NC 27513, USA. SAS Institute, Inc.) and all linear and non-linear regression analysis were conducted with SigmaPlot 11.0 (Systat Software, San Jose, CA).

3 | Results

At constant temperatures, the highest germination percentages for all populations were obtained between 15 ºC and 25ºC (Table 2, Test 1), being maximum at 20ºC. Seeds from Seville population had significantly different germination response comparing to the rest of the populations. A decrease in the germination percentage was observed at much lower (10ºC and 5ºC) or higher (30ºC) constant temperatures (Table 2). Each population was affected similarly by the gradient of temperatures assessed, but the observed germination percentage was different depending on the biotype. Statistical differences were found between populations and incubation temperatures at all constant temperatures ($P < 0.001$) (Test 1, Table 2). At constant 5ºC, the population from Lleida showed >25% of germination, value significantly higher than those observed in the population from Badajoz (1.3%), Seville (2.9%) and Bahía Blanca (9.6%). At constant 10ºC, the populations from Lleida and Bahía Blanca showed significantly higher germination percentages (82.1% and 86.3%, respectively) than the population from Seville (48.8%) and Badajoz (55.4%). At 20ºC, the population from Seville showed the lowest germination percentage (81.3%) significantly different from the rest of the populations, with values between 99.2% for Bahía Blanca and 98.3% for Lleida population. On the other hand, the population from Badajoz showed the highest germination percentage (25.0%) at constant 30ºC, with values significantly different from the rest of the populations. Finally, no germination was observed at 35ºC in any of the populations.

With respect to test 2, the seed exposure to a constant T_{osc} and at different T_m significantly affected the germination percentage. Statistical differences were found between populations and incubation temperatures at all constant temperatures ($P <$

0.001) except for 20–30ºC (P < 0.397). Higher germination percentages were observed at 20–30ºC for populations from Lleida and Seville, 5–15ºC for Badajoz and 15–25ºC for Bahía Blanca, with germination percentages higher than 94% for all of them (Test 2, Table 2). At 10–20ºC and at 5–15ºC, the population from Seville showed lower germination percentages (58.3% and 64.6%, respectively) than the rest of the populations, which always showed values above 82%. At 15–25ºC, the population from Bahía Blanca showed a 98.3%, with significant different to those observed in population from Seville (76.7%) and Badajoz (87.9%) but not with Lleida (97.1%). At 25–35ºC, the population from Badajoz showed the highest germination percentage (45.0%), which was significantly higher than the populations from Lleida (21.3%), Seville (19.6%) and Bahía Blanca (10.0%).

In the case of test 3, where a same T_m and different T_{osc} was assessed, no significant differences in germination percentage were observed inside each population (P = 0.327 for Lleida, P = 0.780 for Badajoz, P = 0.334 for Seville and P = 0.09 for Bahía Blanca), but there were differences between the different T_{osc} considered (P < 0.001), (Test 3, Table 3). At all temperatures assayed, significantly lower germination percentages were observed between populations from Seville (with percentages between 71.7% and 85.07%) and the rest of the populations.

The effect of temperature did not only affect the final germination percentage, but also the germination timing and rate (Figure 1). The cumulate germination of all populations at all temperatures successfully fitted to log–logistic function, except for those temperatures at which germination was too low or null (Table 4). At the lowest and highest constant temperatures, lower values were estimated for parameter x_o, indicating a delay in germination (Table 4; Figure 1). This behaviour is similar in all the populations. In general, the germination rate, identified as parameter b (Table 4) was

faster between 15ºC and 25ºC, compared to at 5ºC, 10ºC and 30ºC, except for the population from Badajoz. Parameter b could not be significantly fitted (P<0.05) for populations from Lleida and Bahía Blanca at 20 ºC and 25ºC, due to the fast germination rate, though the log-logistic function was significantly fitted (Table 4).

3.1 | Estimation of T_b, T_o and T_c

Lorentzian function was significantly adjusted to the $1/d_{50}$ values (Figure 2) for every population with high accuracy (R^2 > 0.86). The optimal temperature (T_o) for populations from Lleida and Seville was established at 21.7ºC, whereas these values were higher for Bahía Blanca and Badajoz: 22.2ºC and 22.3ºC, respectively (Table 4).

Regression lines were successfully fitted for the estimation of the T_b and T_c, with R^2 values ranging between 0.90 and 0.99 (Figure 3) in seven of the eight cases, and being R^2 = 0.81 for the T_c of the Badajoz population. The lowest T_b (4.9ºC) was estimated for the population from Lleida (Table 4), while the highest values (8.9ºC and 8.4ºC) were obtained for populations from Seville and Badajoz, respectively. An intermediate T_b value (6.9ºC) was observed in the Bahía Blanca population (Table 4). The lowest T_c value was also obtained from the Bahía Blanca population (31.5ºC), followed by that from Seville (31.7ºC) and Lleida (32.3ºC). Finally, the highest value was observed in the Badajoz population (34.0ºC). Statistical differences were found between populations (P < 0.001) for T_b but not for T_o and T_c (P < 0.246 and P < 0.103, respectively).

4 | Discussion

All populations showed similar germination behaviour. Germination percentage was highest near the optimum temperature and there were significant differences in the final germination percentages, which appeared mainly at lower temperatures. These differences could be explained, in part, by the climate of the original localities, but also by a possible maternal effect. The environmental conditions under which the mother plant produced the seeds, and also the position of the seed in the plant can impact seed germination. Likewise, water deficit, the age of the plant, the day length, the parental photo-thermal environment, light quality, altitude, and temperature are known, among other factors, to affect germinability (and dormancy in some cases) in other species (Gutterman, 2000; Menegat et al., 2018).

4.1 | Effect of temperature on germination

For all populations, the maximum germination percentage was reached near 20ºC. The estimation of the optimal temperature (T_o) (21.7ºC to 22.3ºC) allowed for little distinction between populations (Table 4). When the temperatures moved away from the optimal, the final germination percentage decreased. This decrease was faster for supra-optimal than for sub-optimal temperatures (Table 2, Test 1). These results are similar to those found by Hardegree (2006) for various gramineous species.

The alternate temperatures did not have a significant effect when the mean temperature was close to the optimum (20ºC). Similar trend was observed by Ottavini et al. (2019) with *C. canadensis*, who did not observed significant differences between constant and alternate temperatures of 15ºC, 20ºC and 25ºC on average. Moving away from the optimal, the alternate temperatures (Table 2, Test 2) favoured the germination of the seeds compared to the corresponding constant mean temperature (Test 1, Table 2). For example, at 10ºC, an oscillation of 10ºC (5–15ºC) increased the total

germination percentage in Lleida, Badajoz and Seville, in between 8% and up to 39%. The Bahía Blanca population was apparently the only one not affected by the oscillation at such low temperature. Similarly, at constant temperatures of 30ºC, germination percentage was, on average, 13.3%; whereas at oscillation, the germination percentage was 24% at 25–35ºC. This increasing germination percentage is also observed by Vidal et al. (2007) when comparing constant 25ºC to 20–30ºC, which increased from less than 50% up to nearly 85%. Differences could be explained by alternate temperatures, which enhance germination in photoblastic species (Roeder et al., 2013) such as *C. bonariensis*. Moreover, temperature changes are more pronounced at the soil surface where *C. bonariensis*, a very small seeded species, germinates better. Furthermore, the germination of this species is null at depths deeper than 2 cm (Wu et al., 2007), similar to *C. canadensis* which germination is reduced to 0% between 0.5 cm and 1cm (Ottavini et al., 2019).

In the intervals with constant mean temperatures and variable oscillation (Table 2, Test 3), there are not differences between the intervals and with the constant 20ºC, with similar percentage germination with the intervals with variable mean and constant oscillation when the mean is close to 20ºC.

The differences observed between germination percentages at constant and alternate temperatures could correspond to depth- and gap-sensing mechanism: temperature oscillations are more pronounced at or near to the soil surface and the amplitude of these fluctuations decreased with burial depth (Ren et al., 2002). This could be an adaptation that staggers the germination with the changing temperatures throughout the seasons (Vidal et al., 2007).

4.2 | Effect of the origin of the population on germination

Differences in germination percentages are accentuated between populations (Table 2). The population from Lleida, which is a comparatively colder location (Table 1), showed more germination (26.7%) at the lowest temperature assayed (5ºC). The populations from warmer climates had lower germination percentage at this temperature (1.3% and 2.9% for Badajoz and Seville, respectively). These differences are not so clear when comparing the populations at 30ºC, wherein germination percentages from Badajoz and Seville populations showed statistical differences of 25.0% and 7.1%, respectively.

Under the 10ºC oscillation treatment (Table 2, Test 2), excluding the higher one (25/35ºC), there were a high germination percentages and significant differences between the intervals across the populations. Similarly, there were high germination percentages for seeds subjected to temperature oscillations that were 20ºC on average (Table 2, Test 3).

Higher germination percentages with 10ºC oscillation occurred between 15/25ºC and 20/30ºC in all populations, except for that of Badajoz which had highest germination percentages at 5/15ºC. Except for Seville population, these results are not in accordance with those from Travlos & Chachalis (2013), who found differences between 15/25ºC and 20/30ºC in populations of *C. bonariensis* from Greece. On the other hand, the lack of differences between 10/20ºC and 15/25ºC intervals in populations from Lleida and Badajoz agrees with other authors finding that there is less variation for these temperature ranges in climatically closer biotypes. Despite this, the Spanish populations from Lleida and Badajoz seem to be more adapted to colder winters (exhibiting more winter-summer oscillation) than those from Greece, as at 5/15ºC the Spanish ones obtained over 90% germination, while the Greek ones did not exceed 35%.

Our results also agree with those from Karlsson & Milberg (2007), for populations from Ethiopia, Mexico and Morocco, for the 10/20, 15/25 and 20/30ºC intervals, but not for the 5/15ºC, where Lleida, Badajoz and Bahía Blanca populations obtained higher germination percentages (82.1-94.6%) than in previous studies (below 75%). The low germination percentages obtained by Karlsson & Milberg (2007) at 5/15ºC could be explained by the local climatic conditions of these populations, which are classified as: Tropical pluviseasonal (Mexico), Tropical xeric (Ethiopia), and Mediterranean xeric-oceanic (Morocco) (Rivas-Martinez & Rivas-Saenz, 1996-2018). Population differences could be due to the adaptation to the climatic characteristics of each original site where the seeds were produced (Clements & DiTommaso, 2011), as reported for *C. canadensis* (Tozzi et al., 2014).

4.3 | Germination patterns and threshold values

The germination patterns of all population at all constant temperatures were, in general, successfully fitted to a log-logistic sigmoidal function (Table 3, Figure 1). The lack of this adjustment in some cases (Table 3) could be partially explained by the exceedingly high or low germination rates of the populations: 20ºC and 25ºC for Lleida and Bahía Blanca, and 5ºC and 20ºC for Badajoz and Seville.

The calculation of the x_0 with the log-logistic model allowed the estimation of the threshold values of T_b and T_c (Figure 3), while T_o was estimated with the Lorentzian model applied to the total germination percentages (Figure 2). Our results for the population from Lleida agree with Wu et al. (2007), which estimated that *C. bonariensis* can germinate between 4.2ºC and 30ºC, even if the T_o is 20ºC. The T_b of one of our populations (Lleida) are close to that from Wu et al. (2007), and the values are in accordance with the germination response explained above: T_b in Lleida shows

the lowest value (4.9ºC) followed by Bahía Blanca (6.9ºC), which agrees with their local climatic origin. In accordance with this, the T_b in Seville (8.9ºC) and Badajoz (8.4ºC) are higher than in Lleida and Bahía Blanca, and not considerably different from that estimated by Zambrano-Navea et al. (2013) (10.6ºC). The differences in the T_b from Lleida and Bahía Blanca (4.9ºC and 6.9ºC) compared to the T_b obtained by Zambrano-Navea et al. (2013) can be explained by the variations in the experimental design, as these authors used constant 15ºC as the lowest temperature, while we also experimented with 10ºC and 5ºC, which obtained 48.8% and 2.9% germination, respectively. The idea that *C. bonariensis* is a summer weed could have led to the thought that its T_b was similar to other summer weeds, such as *Amaranthus retroflexus* which is estimated to be between 10.0ºC and 12.9ºC (Loddo et al., 2018), or *C. canadensis*, which is between 8ºC and 14ºC, depending on the population (Tozzi et al., 2014).

The estimated ceiling temperature (T_c) varied from 31.5ºC to 34.0ºC, which agrees with the lack of germinated seeds at constant 35ºC in any population. These results also agree, in part, with those from Yamashita & Guimaraes (2011), who only obtained 6% germination at a constant 35ºC and 1% germination at 40ºC. Similar to the tendency in the other threshold values, the lowest T_o was obtained in the population from Lleida, but also in Seville (21.7ºC), while the highest one was obtained in the population from Badajoz (22.3ºC). These T_o values, which could in general be established at about 22 ºC (±0.3ºC), differ from that selected from the literature (Wu et al., 2007) to set the Test 2 and Test 3 of the experiment, and could be considered for future experiments.

4.4 | Implications for developing emergence models

The emergence model for *C. bonariensis* from Zambrano-Navea *et al.* (2013) has been proven to be valuable in several sites. This model was developed and validated with populations from the South of Spain, and has less relevance for other climatic biotypes and regions since the germination behaviour and the threshold values of other populations are different. The genetic variation of the *Conyza* species (Ming-Xun et al., 2010) could explain, in part, these results. Even if *C. bonariensis* has the ability to spread and disperse its seeds long distances (Savage et al., 2014), which could diminish the variations between populations, these differences are still important according to the results of the present and past work (Karlsson & Milberg, 2007). In addition to genetic origin, the maternal effect is another factor which could have enhanced differences between local populations.

In our study, there were four degrees (ºC) of difference in T_b between the seeds from different origins, thereby impeding the development of a common model. In order to develop a model that could be widely applied, the next step is to test differences in the base parameters and germination behaviour of populations coming from different geographical sites, but belonging to the same climatic biotype. If there are not any differences between them, a more precise model could be developed or the current one created by Zambrano-Navea et al. (2013) could be readjusted to the populations of a certain climatic area.

5 | Conclusion

The germination percentage of *C. bonariensis* was higher when close to the optimal temperature obtained (22ºC), both for constant and alternate temperatures. In the intervals with same T_{osc} and different T_m, some obtained higher germination

percentages than at constant temperature. In the intervals with different T_{osc} but the same T_m, there are high germination percentages for all temperatures, without significant differences from the constant temperature. The different populations responded to the tests according to the apparent influence of their climatic origin. The biotype adapted to the coldest winter site (Lleida) had more germinated seeds at lower temperatures, while the biotypes adapted to warmer climatic sites (Badajoz and Seville) were more acutely affected by a temperature decrease. The differences in the threshold values for the cardinal parameters, as well as the in the germination behaviour of the different climatic biotypes prevents us from developing a common germination/emergence model. Thus, there is further need of investigation to achieve the goal of obtaining accurate models for each climatic region.

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Conflicts of interests

Authors declare that there are not conflicts of interest.

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Table 1. Climatic characterization of the origin locality of each population. T_{Mean}: Annual Mean Temperature; T_{Warm}: Mean temperature of warmest month; T_{Cold}: Mean temperature of coldest month

Climatic characterization							
Location	Macro Bioclimate	Ombrotype	Thermotype	Bioclimate	T_{mean}	T_{warm}	T_{cold}
Lleida	Mediterranean	Xeric	Mesomediterranean	Continental	15.0	25.2	5.5
Badajoz	Mediterranean	Xeric	Mesomediterranean	Oceanic	17.1	26.1	8.6
Seville	Mediterranean	Xeric	Thermomediterranean	Oceanic	19.2	28.2	10.9
Bahía Blanca	Temperate	Xeric	Mesotemperate	Oceanic	15.3	23.6	7.5

Temperature means calculated for the period 1983–2010 for the Spanish locations and 1981–2010 for the Argentinian

Table 2. Total percentage of germination for the *Conyza bonariensis* populations at each experiment. Test 1, constant temperatures; Test 2, alternate temperatures (variable mean/constant oscillation); Test 3, alternate temperatures (constant mean/variable oscillation).

	T (ºC)	Lleida	Badajoz	Seville	Bahía Blanca	SED	LSD	d.f.
Test 1	5	26.7	1.3	2.9	9.6	4.93	9.81	31
	10	82.1	55.4	48.8	86.3	8.98	18.39	31
	15	92.5	86.3	74.6	97.1	6.48	13.27	31
	20	98.3	96.3	81.3	99.2	3.12	6.40	31
	25	93.3	90.0	77.9	99.2	3.35	6.87	31
	30	15.8	25.0	7.1	5.4	3.78	7.75	31
	35	0.0	0.0	0.0	0.0	-	-	-
	SED	5.80	5.44	5.09	3.78			
	LSD	11.60	10.92	10.23	7.58			
	d.f.	55	55	55	55			
Test 2	5–15	90.0	94.2	64.6	82.1	5.84	11.96	31
	10–20	92.5	83.3	58.3	89.2	5.90	12.09	31
	15–25	97.1	87.9	76.7	98.3	4.55	9.32	31
	20–30	98.3	93.3	95.0	94.6	2.99	6.12	31
	25–35	21.3	45.0	19.6	10.0	5.07	10.38	31
	20	98.3	96.3	81.3	99.2	3.12	6.40	31
	SED	2.98	4.77	6.49	3.94			
	LSD	6.01	9.63	13.10	7.96			
	d.f.	47	47	47	47			
Test 3	18–22	98.3	93.8	83.3	94.6	3.74	7.65	31
	16–24	97.1	90.8	71.7	94.6	5.10	10.45	31
	14–26	95.0	94.2	85.0	99.2	4.14	8.49	31
	12–28	99.1	93.3	79.6	100.0	4.09	8.38	31
	10–30	93.8	95.4	75.8	92.5	5.12	10.48	31
	20	98.3	96.3	81.3	99.2	3.12	6.40	31
	SED	2.76	3.79	6.45	3.11			
	LSD	5.57	7.64	13.01	6.28			
	d.f.	47	47	47	47			

SED, standard error of the difference between two means; LSD, least significant difference between two means at $P = 0.05$; d.f., degrees of freedom associated with LSDs and SEDs.

Table 3. Values of parameters for the log-logistic models obtained from the cumulative germination data for each population assessed at every constant temperature.

Population	T(ºC)	a	b	x_0	F	P
Lleida	5	26.9957	1.2307	16.6264	3702.9921	<0.0001
	10	80.9581	0.7598	9.7044	7866.2978	<0.0001
	15	91.4461	0.6621	2.5887	366.9728	<0.0001
	20	98.3114	0.0724*	1.7998*	502481.464	<0.0001
	25	93.0921	0.0911*	1.7858*	13097.5736	<0.0001
	30	15.4061	2.4003	4.7235	165.6618	<0.0001
Badajoz	5	1.7422*	1.2499*	19.5611	135.2574	<0.0001
	10	53.6136	0.9767	11.4118	1659.4462	<0.0001
	15	85.6338	1.2452	3.9909	264.9829	<0.0001
	20	96.1187	0.1165*	1.8273	28831.9755	<0.0001
	25	89.1273	0.2082	1.8518	918.8929	<0.0001
	30	24.2102	2.8306	4.6569	98.0223	<0.0001
Seville	5	2.6267	0.2815*	17.0325	1638.7769	<0.0001
	10	48.6567	1.2155	12.6461	2207.0097	<0.0001
	15	74.5380	1.1501	5.0842	2116.4081	<0.0001
	20	80.2973	0.2188*	1.8141	463.9906	<0.0001
	25	77.5177	0.4436	2.7171	2614.6267	<0.0001
	30	7.1728	2.9543	5.2191	143.8073	<0.0001
Bahía Blanca	5	13.5797	2.0785	19.1641	1058.6897	<0.0001
	10	84.7909	0.8799	10.6539	4965.3598	<0.0001
	15	96.5026	0.8747	3.4091	459.5015	<0.0001
	20	99.1229	0.1018*	1.8307	129376.9258	<0.0001
	25	98.9696	0.1173*	1.8312	19924.4810	<0.0001
	30	5.6035	2.4995	9.5902	415.4343	<0.0001

* Parameters not fitted (P<0.05) without incidence in the log-logistic function.

Table 4. Estimated base temperatures (T_b), optimal temperature (T_o), and ceiling temperatures (T_c), for each population of *Conyza bonariensis* assessed. Mean values are presented in ºC.

	Lleida	Badajoz	Sevilla	Bahía Blanca	SED	LSD	d.f.
T_b	4.9	8.4	8.9	6.9	0.45	0.92	31
T_o	21.7	22.3	21.7	22.2	0.31	0.64	31
T_c	32.3	34.0	31.7	31.5	1.05	2.17	27

SED, standard error of the difference between two means; LSD, least significant difference between two means at $P = 0.05$; d.f., degrees of freedom associated with LSDs and SEDs.

Figure 1. Cumulative germination percentage (%) of *Conyza bonariensis* at constant temperatures of 5ºC (●), 10ºC (○), 15ºC (▼), 20ºC (Δ), 25ºC (■), 30ºC (□) across the different populations.

Figure 2. Lorentzian functions adjusted to the $1/d_{50}$ values obtained at each temperature by each population assessed. R^2 of the functions and RMSE are provided.

Figure 3. Estimation of the base temperatures (T_b) on the left and ceiling temperatures (T_c) on the right for each of the *Conyza bonariensis* populations. Regression lines are presented, together with the lineal functions for each T_b and T_c, followed by the R^2.